

# **Dihydrorhodamine 123**

Cat. No.: AFG-MCH-00004

CAS No.: 109244-58-8 Molecular Formula:  $C_{21}H_{18}N_2O_3$  Molecular Weight: 346.38 Emission (Em): 536

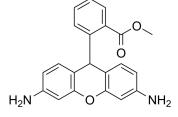
Target: Fluorescent Dye

Pathway: Others

Storage: -20°C, protect from light

515

\* The compound is unstable in solutions, freshly prepared is recommended.



#### **SOLVENT & SOLUBILITY**

In Vitro

Excitation(Ex):

DMSO: 100 mg/mL (288.70 mM; Need ultrasonic)

| Preparing<br>Stock Solutions | Solvent Mass<br>Concentration | 1 mg      | 5 mg       | 10 mg      |
|------------------------------|-------------------------------|-----------|------------|------------|
|                              | 1 mM                          | 2.8870 mL | 14.4350 mL | 28.8700 mL |
|                              | 5 mM                          | 0.5774 mL | 2.8870 mL  | 5.7740 mL  |
|                              | 10 mM                         | 0.2887 mL | 1.4435 mL  | 2.8870 mL  |

Please refer to the solubility information to select the appropriate solvent.

In Vivo

1. Add each solvent one by one: 10% DMSO >> 90% (20% SBE- $\beta$ -CD in saline) Solubility: 2.5 mg/mL (7.22 mM); Suspended solution; Need ultrasonic

## **BIOLOGICAL ACTIVITY**

Description

Dihydrorhodamine 123 (DHR 123) is a non-fluorescent reactive oxygen species (ROS) indicator. Dihydrorhodamine 123 is oxidized to fluorescent Rhodamine 123 (HY-D0816) within cells in the presence of reactive oxygen species and it localizes in mitochondria.

In Vitro

In the presence of 10  $\mu$ M Dihydrorhodamine 123 (DHR 123) the stimulation of the neutrophil NADPH oxidase by the addition of 50 nM phorbol 12-myristate 13-acetat (PMA) resultes in an increase in the rate of rhodamine generation. The fluorescent intensity of the cells, in the presence of 10  $\mu$ M Dihydrorhodamine 123, increases with time following the addition of 50 nM PMA. In the presence of 10  $\mu$ M Dihydrorhodamine 123, induced HL60 cells show a sustained increase in fluorescence following the addition of 50 nM PMA $^{[1]}$ .

AffiGen has not independently confirmed the accuracy of these methods. They are for reference only.

#### **PROTOCOL**

#### Cell Assay [1]

The HL60 cells are incubated at  $6\times10^6$  cells/mL in Krebs-Ringer buffer at  $37^\circ\text{C}$  containing  $10~\mu\text{M}$  Dihydrorhodamine 123 (DHR). The generation of  $O_2^-$  is initiated by the addition of 50 nM phorbol 12-myristate 13-acetat (PMA) and the progress of the generation of rhodamine 123 is monitored in  $50-\mu\text{L}$  aliquots ( $3\times10^5$  cells) diluted tenfold before analysis. The uninduced HL60 cells are loaded with  $5~\mu\text{M}$  carboxy SNARF-1 AM acetate (SNARF-AM) in the Na<sup>+</sup> medium for 10 min at  $37^\circ\text{C}$  and washed by centrifugation and resuspension to remove unhydrolysed SNARF ester<sup>[1]</sup>.

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## CUSTOMER VALIDATION

- Adv Mater. 2025 Jan 26:e2410992.
- · Adv Funct Mater. 2025 Jan 16.
- ACS Nano, 2025 Jan 7.
- Small. 2024 Jan 14:e2306916.
- Adv Healthc Mater. 2024 Sep 3:e2402079.

#### **REFERENCES**

[1]. Lydia M. Henderson et al. Dihydrorhodamine 123: a fluorescent probe for superoxide generation? Eur. J. Biochem. 217, 973-980.



## For ordering and technical support:

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